

Divergent Selection for Phosphorus Concentration in Reed Canarygrass

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ABSTRACT

Phosphorus is a vital nonrenewable natural resource that is essential for plant and animal growth. Grass swards and buffer strips can be used to reduce P losses that occur by soil erosion, surface runoff, or leaching. The objective of this study was to conduct one cycle of divergent selection for P concentration in reed canarygrass (*Phalaris arundinacea* L.) and to evaluate the effects of selection on P concentration, forage yield, and P uptake (the product of P concentration and forage yield). Divergent selections and original populations were evaluated in sward plots, harvested three times per year for 2 yr at three locations. Selection responses indicated that P concentration is a heritable trait in reed canarygrass; that genetic gains were highly repeatable across harvests, locations, and years; and that there is a negative genetic correlation between P concentration and sward-plot forage yield. Future selection efforts must be based on genotypic selection methods, in which half-sib families are evaluated for forage yield, P concentration, and P uptake in sward plots.

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Abbreviations: ICPE, inductively coupled plasma emission; NIRS, near-infrared reflectance spectrophotometer; OM, organic matter; PLS, pure live seed; SEP(C), standard error of prediction (calibration).

PHOSPHORUS IS A VITAL nonrenewable natural resource that is essential for plant and animal growth. Judicious and efficient management of P is necessary to maintain profitable and sustainable crop and livestock production systems and to protect surface-water resources (Frossard et al., 2000; Sharpley et al., 2000). Phosphorus can be lost from agricultural fields to surface or groundwater by soil erosion, surface runoff, and leaching (Higgs et al., 2000). Loss of P in surface runoff occurs in both sediment-bound and dissolved forms, the latter having greater and more immediate biological reactivity (Sharpley, 1998; Uusi-Kämpä et al., 2000).

Grass swards and buffer strips can be used to reduce P losses by each of the above three mechanisms. Soil erosion from well-managed permanent or continuous grassland is virtually nonexistent (Wadleigh et al., 1974). Perennial grasses trap P from surface runoff by sedimentation of P-enriched soil particles and

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uptake of dissolved reactive P (Heathwaite et al., 1998; Sharpley, 1998; Uusi-Kämpä et al., 2000). Used as buffer strips, grasses reduce P losses by reducing both total runoff and the suspended solids in runoff waters (Borin et al., 2004; Patty et al., 1997). The “hydraulic roughness” of dense grass crops decreases overland flow velocity and sediment transport capacity (Baker, 1992; Dhillon et al., 1989). Removal of P-enriched herbage is an effective means of “mining” dissolved reactive P both from recent runoff events (Sharpley, 1998; Uusi-Kämpä et al., 2000) and from the soil profile (Sistani et al., 2003). Because P is readily utilized by ruminant livestock, total P in forages is a reasonable and acceptable measure of P availability for livestock (Galdámez-Cabrera et al., 2004).

Because P is a finite natural resource and one of the most common mineral supplements for both ruminant and nonruminant livestock (Valk et al., 2000), P reserves in production fields should be considered a valuable on-farm resource. Production fields vary in P reserves and in their susceptibility to P loss, suggesting that the most effective nutrient budgeting should be conducted at the field level (Higgs et al., 2000). Perennial grass swards may be used on different scales, from small patches or buffer strips to the farm level, to minimize P loading of surface waters and to preserve on-farm P reserves for use in developing more efficient and cost-effective on-farm nutrient budgets.

Perennial grasses with improved P uptake would be desirable for more rapid reduction of soil P reserves, improving the potential to mitigate P losses via subsurface flow or leaching. Phosphorus uptake may be enhanced by increases to either or both dry matter forage yield and P concentration of the herbage. An increase in P concentration would outwardly appear to have greater value than an increase in forage yield, because a P-enriched forage would also serve as a replacement for importing P supplements onto the farm, decreasing both input costs and reliance on nonrenewable natural resources. Both P concentration and forage yield are genetic traits that are heritable in a wide range of species (Casler et al., 1996; Vose, 1963). The objective of this study was to conduct one cycle of divergent selection for P concentration in reed canarygrass (*Phalaris arundinacea* L.) and to evaluate the effects of selection on P concentration, forage yield, and P uptake.

MATERIALS AND METHODS

A selection nursery of reed canarygrass was established from 70-d-old plants in May 1997 at Arlington, WI, on a Plano silt loam soil (fine-silty, mixed, superactive, mesic Typic Argiudoll). Each plant was raised in a greenhouse from an individual seed. The selection nursery consisted of a total of 1800 plants, 40 plants each from five cultivars and 45 polycross families of the population WR00, all classified as low in gramine and free of tryptamine and β -carboline alkaloids (Casler and Undersander, 2006). The experimental design was a randomized complete

block with four replicates and each plot consisting of a row of 10 plants from a population. All plants were planted on a 0.9-m spacing in both directions. Plants were fertilized with 56 kg N ha⁻¹ before each establishment-year growth cycle, and weeds were controlled by a combination of hand weeding and pre-emergence herbicides throughout the duration of the experiment (Falkner and Casler, 1998). Soil samples were collected before transplanting, dried, and analyzed for pH, organic matter (OM) by loss-on-ignition (Combs and Nathan, 1998), and P and K by Bray and Kurtz P-1 extract (Frank et al., 1998; Peters, 2006). Soil fertility measurements of the selection nursery were 6.7 pH, 29 g kg⁻¹ OM, 47 g kg⁻¹ P, and 140 g kg⁻¹ K.

All plants were harvested in early spring and midsummer 1998 and early spring 1999. The spring harvest occurred when plant canopies were approximately 20-cm tall. Following this harvest, plants were allowed to grow until anthesis, when they were cut and allowed to regrow. The summer harvest occurred when the average plant canopy was again approximately 20-cm tall, generally in mid- to late July. Plants were fertilized with 90 kg N ha⁻¹ in early spring and at the initiation of their regrowth cycle in each year. Plants were harvested by hand at a cutting height of 9 cm. All harvested plant samples consisted entirely of leaf blades and sheaths to avoid confounding results with plant-to-plant differences in leaf-stem ratio (Casler, 1999). Samples were stored in paper bags and dried at 60°C. All plants were rated for vigor (visual estimate of aboveground biomass) at the time of harvesting, using a rating scale of 1 = too small to sample, to 9 = most vigorous plant in the nursery at the time of rating. Many plant samples with extremely low vigor were too small to include in the forage analyses.

Dry samples were ground through a 1-mm screen of a Wiley-type mill and scanned on a near-infrared reflectance spectrophotometer (NIRS) (FOSS model 6500 and ISI software, FOSS North America, Eden Prairie, MN). A subset of 60 reed canarygrass samples plus 70 orchardgrass (*Dactylis glomerata* L.) samples collected from an adjacent experiment managed in a similar manner was chosen for calibration by NIRS software using cluster analysis of reflectance spectra (Shenk and Westerhaus, 1991). The number of samples was based on previous experience to allow an adequate and complete sampling of variability within the population of samples. Two species were used because these calibrations represented the initial attempts to develop broad-based grass calibrations for multiple traits and multiple species. Total phosphorus concentration was determined by nitric acid digestion of 0.5-g samples and analysis by inductively coupled plasma emission (ICPE) spectroscopy (Peters, 2006). Calibration statistics for the NIRS equation were $R^2 = 0.81$ and standard error of prediction (calibration) [SEP(C)] = 0.561 g kg⁻¹. Due to the size of the experiment and the practical limitations of using ICPE spectroscopy on such a large experiment, the NIRS calibration was used to predict P concentration for all samples. We recognize that NIRS has limitations in predicting mineral elements, largely due to its inability to recognize chemical bonds associated with most mineral elements, but there are a number of examples of successful NIRS calibrations for P concentration and other mineral elements in the literature (Fahey and Hussein, 1999; Halgerson et al., 2004; Lu et al., 2006; Vasquez de Alana et al., 1995).

Data on vigor and P concentration were analyzed by generalized least squares ANOVA, assuming all effects to be random (harvests, blocks, populations, and plants). The ANOVA model was a split-plot-in-time with separate error terms for four portions of the analysis: (i) harvests, (ii) cultivars and families (populations), (iii) all interactions of populations with harvests, and (iv) plants within populations (Steel et al., 1997). Broad-sense heritability, the proportion of phenotypic variance due to variation among population means, was estimated for populations as $H = s^2_p / [s^2_p + (s^2_{PB}/b) + (s^2_{PH}/h) + s^2_e/rh]$, where s^2_p , s^2_{PB} , s^2_{PH} , and s^2_e are the variance components for populations (P), populations \times blocks (PB), populations \times harvests (PH), and error (e), respectively, b = number of blocks, and h = number of harvests. Repeatability of P concentration on an individual-plant basis (the consistency of multiple P measurements across harvests and years) was estimated as the intraclass correlation coefficient, $r_1 = s^2_{P/P} / (s^2_{P/P} + s^2_{WP})$, where $s^2_{P/P}$ = the variance component for plants within populations and s^2_{WP} = the variance component for harvests \times plants within populations [Error(d)].

Plants with any single-harvest vigor rating of ≤ 5 were eliminated from contention, leaving 733 reed canarygrass plants. Mean P concentration over three harvests was computed for all remaining plants. Ten plants were selected from among the remaining plants, the five plants with the highest mean P concentration and the five plants with lowest mean P concentration. Low-P selections originated from five different polycross families, and high-P selections originated from one cultivar (Venture) and three polycross families.

These 10 plants were split into four ramets each and transplanted into two isolated crossing blocks in July 2000 (high P and low P). Crossing blocks and the original nurseries were fertilized with 56 kg N ha⁻¹ in spring 2001. Seed was harvested on each plant of each crossing block and on all plants of the original selection nursery in July 2001. All panicles were harvested from each plant of the four replicated crossing blocks. Seeds were threshed, cleaned, and bulked in equal mass for all plants within a crossing block. Two panicles were harvested from each plant in the original selection nursery, and these were formed into one bulk lot before threshing and cleaning.

All three seed lots were tested for germination in autumn 2001 (Association of Official Seed Analysts, 1998). Plots of each population were established at Arlington, Lancaster, and Marshfield, WI, in April 2002. Soil types were Plano silt loam at Arlington, Fayette silt loam (fine-silty, mixed, superactive, mesic Typic Hapludalf) at Lancaster, and Withee silt loam (fine-loamy, mixed, superactive, frigid Aquic Glossudalf) at Marshfield. Each population was planted on a pure-live-seed (PLS) basis at a seeding rate of 1500 PLS m⁻², which is equivalent to an average of 13 kg ha⁻¹. The experimental design was a randomized complete block with eight replicates. Plots were 0.9 \times 3.0 m and consisted of five drilled rows, spaced 15 cm apart. Plots were clipped twice during the establishment year to control annual weeds. Soil samples were collected before planting, dried, and analyzed for pH, OM by loss-on-ignition (Combs and Nathan, 1998), and P and K by Bray and Kurtz P-1 extract (Frank et al., 1998; Peters, 2006), the results of which are presented in Table 1.

Table 1. Soil-test data for three Wisconsin locations before establishment of field experiments in 2002.

Location	pH	OM [†] g kg ⁻¹	P [†] mg kg ⁻¹	K [†] mg kg ⁻¹
Arlington	6.5	30	45	123
Lancaster	7.3	28	26	141
Marshfield	7.0	34	36	94

[†]OM = organic matter, determined by loss-on-ignition (Combs and Nathan, 1998); P and K determined by Bray and Kurtz P-1 (Frank et al., 1998; Peters, 2006).

Plots were fertilized with 90 kg N ha⁻¹ in spring 2003 and 2004 and following first and second harvests in each year. Plots were harvested with a flail-type harvester in early June (post-heading), early August, and late October of 2003 and 2004, with the exception of third harvest at Lancaster 2003, which was eliminated due to severe drought stress. A 500-g sample was collected from the cut forage of each plot for dry matter and laboratory analysis. Samples were dried at 60°C and used to adjust forage yield to a dry-matter basis. Dry samples were ground through a 1-mm screen of a Wiley-type mill and scanned on a NIRS. A subset of 70 samples was chosen for calibration by NIRS software using cluster analysis of reflectance spectra (Shenk and Westerhaus, 1991). Calibration samples were analyzed for P, N, K, Ca, and Mg concentration by standard methods. Nitrogen was analyzed by macro-Kjeldahl digestion of a 1-g sample, followed by distillation and titration (Peters, 2006). For the remaining elements, a 0.5-g sample was dry ashed at 500°C, dissolved in HCl, and analyzed either colorimetrically (P) or by atomic absorption spectroscopy (K, Ca, Mg) (Peters, 2006). Calibration statistics were $R^2 = 0.83$ and SEP(C) = 0.437 g kg⁻¹ for P, $R^2 = 0.99$ and SEP(C) = 1.053 g kg⁻¹ for N, $R^2 = 0.93$ and SEP(C) = 2.237 g kg⁻¹ for K, $R^2 = 0.75$ and SEP(C) = 0.534 g kg⁻¹ for Ca, and $R^2 = 0.63$ and SEP(C) = 0.469 g kg⁻¹ for Mg.

Data for each harvest and the weighted mean across harvests were analyzed separately by generalized least squares ANOVA using the split-plot-in-time model (Steel et al., 1997). Years, locations, and blocks were assumed to be random effects, and populations was a fixed effect. The linear effect of selection and the asymmetrical effect of selection were computed as contrasts in each ANOVA. Realized heritability for P concentration was computed as $(P_H - P_L) / (S_H - S_L)$, where P_H = mean of the high-P progeny population, P_L = mean of the low-P progeny population, S_H = mean of the high-P parental selections, and S_L = mean of the low-P parental selections (Falconer and Mackay, 1996).

RESULTS

Reed canarygrass families and cultivars differed in mean P concentration in the original selection nursery (Table 2). Plants within reed canarygrass families and cultivars accounted for 11% of the variance of a population mean and had a larger variance component than its error term. Broad-sense heritability of the reed canarygrass population was 0.46 and repeatability of single-plant P concentration was 0.27. Despite the significance of numerous error terms and the population \times harvest interaction, the

Table 2. Analysis of variance for P concentration and plant vigor rating measured on plants in the original reed canarygrass selection nursery.

Source of variation	df	P concentration		Plant vigor	
		MS	VEM†	MS	VEM†
		%			
Harvest	2	0.034146**		399.651**	
Block	3	0.018072*		4.353	
Error(a)	6	0.004036**		19.439**	
Population	49	0.004500**	46	10.258	9
Error(b)	147	0.001839**	31	7.203**	37
Harvest × population	98	0.000866**	3	4.214**	6
Error(c)	277	0.000606**	3	2.689**	7
Plants/population	1162	0.000795**	11	5.144**	36
Error(d)	1287	0.000377	7	0.836	5

*Mean square significant at $P < 0.05$.**Mean square significant at $P < 0.01$.

†VEM = contribution to the variance of a population mean (%).

Table 3. Means and standard deviations of phosphorus concentration for reed canarygrass grown at three Wisconsin locations for 2 yr.

Year	Location	Harvest 1	Harvest 2	Harvest 3	Mean†
		g kg ⁻¹			
2003	Arlington	2.78 ± 0.02	3.07 ± 0.08	2.33 ± 0.03	2.78 ± 0.03
2003	Lancaster	2.97 ± 0.04	3.42 ± 0.03	—‡	3.15 ± 0.03
2003	Marshfield	3.14 ± 0.04	3.02 ± 0.03	2.95 ± 0.05	3.06 ± 0.02
2004	Arlington	3.21 ± 0.06	3.60 ± 0.02	3.69 ± 0.11	3.37 ± 0.05
2004	Lancaster	3.09 ± 0.06	3.71 ± 0.02	3.78 ± 0.06	3.44 ± 0.03
2004	Marshfield	3.23 ± 0.06	3.08 ± 0.02	3.86 ± 0.05	3.32 ± 0.04

†Weighted by forage yield at each harvest.

‡No harvest due to extreme drought.

Table 4. Means and standard deviations of phosphorus uptake for reed canarygrass grown at three Wisconsin locations for 2 yr.

Year	Location	Harvest 1	Harvest 2	Harvest 3	Total
kg ha ⁻¹					
2003	Arlington	6.46 ± 0.19	6.67 ± 0.38	3.26 ± 0.24	16.39 ± 0.61
2003	Lancaster	16.61 ± 0.25	13.48 ± 0.33	— [†]	30.08 ± 0.49
2003	Marshfield	13.54 ± 0.31	12.56 ± 0.49	4.02 ± 0.24	30.11 ± 0.62
2004	Arlington	8.89 ± 0.39	3.17 ± 0.36	3.23 ± 0.38	15.29 ± 0.72
2004	Lancaster	14.33 ± 0.49	11.36 ± 0.32	8.51 ± 0.32	34.20 ± 0.84
2004	Marshfield	11.91 ± 0.27	6.22 ± 0.26	5.90 ± 0.33	24.03 ± 0.36

‡No harvest due to extreme drought.

two genetic components (among and within populations) accounted for 57% of the variance of a population mean for P concentration of reed canarygrass. For vigor ratings, broad-sense heritability of reed canarygrass populations was only 0.10, but repeatability of single-plant vigor ratings was 0.84. Among and within population genetic variances combined accounted for 45% of the variance of a population mean for vigor. Plant vigor and P concentration were not correlated with each other in the original selection nursery ($r = -0.06$ to 0.14).

Phosphorus concentration in the selection–evaluation experiments was moderately variable across loca-

tions, years, and harvests (Table 3). There was a general trend for P concentration to be positively correlated across harvests, and P concentration tended to be consistently highest at all three locations in 2004. Phosphorus uptake (P concentration × forage yield) in the selection–evaluation experiments was much more variable than P concentration across locations, years, and harvests (Table 4). The variability in P uptake was due to variability in forage yield ($r = 0.99$; $P < 0.01$) and not related to variability in P concentration ($r = -0.08$). Location had the largest effect on P uptake; Lancaster was consistently highest in P uptake, while Arlington was consistently lowest in P uptake. Because average P concentration showed little variation among harvests (Table 3), P uptake declined from spring to autumn along with forage yields.

Divergent selection for P concentration was successful in reed canarygrass, with selection responses ranging from 0.21 g kg^{-1} for Harvest 2 (3.8% of the original population mean) to 0.35 g kg^{-1} for Harvest 3 (5.3% of the original population mean) (Table 5). Forage yield of reed canarygrass had a negative correlated response to divergent selection for P concentration for all three harvests. This effect was greatest for the first harvest, gradually declining throughout the summer. As a result of the large reduction in first-harvest forage yield due to selection for high P concentration, P uptake declined with selection for high P concentration at first harvest and as a total across harvests. Selection responses for these three variables were all consistent across locations and years, with the main effect of populations accounting for 60 to 88% of the variance of a population mean (data not shown). While there were some population × location and population × year interactions, they resulted from small deviations in the magnitude of selection responses and had no effect on the overall conclusions.

Correlated selection responses for N, K, and Ca were all positive and generally significant for all three harvests (Table 6). For Mg, only the first-harvest response was significant, but it was also positive. Selection for high P concentration acted to increase the concentrations of all mineral elements measured in this study. Although not all responses were significant, N, K, Ca, and Mg uptake responded to selection in a manner similar to that of P uptake (Table 7). Uptake responses of each element matched those of forage yield, with the magnitude of responses generally largest for first harvest and declining for second and third harvests.

DISCUSSION

The analysis of variance results from the original selection nurseries suggested that divergent selection for P concentration would be effective in reed canarygrass, and we observed this to be the case. Significant genetic variation for herbage P concentration has been demonstrated in a number of perennial grasses, including smooth brome-grass, *Bromus inermis* Leyss. (Casler and Reich, 1987); timothy, *Phleum pratense* L. (Bélanger et al., 2002; Jefferson, 2005); tall fescue, *Lolium arundinaceum* (Schreb) Darbysh. (Sleper et al., 1977); and Russian wildrye, *Psathyrostachys juncea* (Fisch.) Nevski (Asay and Mayland, 1990; Asay et al., 2001). Mixed results were obtained for crested wheat-grass (*Agropyron* spp.), with significant genetic variability for P concentration in a narrow polycross population (Asay et al., 1996), but no genetic variability observed for a broad array of cultivars, parallel to that used in our study (Vogel et al., 1989). Basing our reed canarygrass selection units entirely on leaves clearly did not limit the potential for genetic variability to be expressed, despite the fact that nearly 60% of the total P in forage plants is located in the stem fraction (Pederson et al., 2002).

The ineffectiveness of mild selection pressure for vigor to maintain the forage yield potential of the original population may be due to a low heritability of vigor rating, a low genetic correlation of spaced-plant vigor with sward-plot forage yield, a negative genetic correlation between P concentration and forage yield, or a combination of these. Low heritability would exacerbate the effects of drift, allowing random changes in allele frequency or random loss of alleles to be more important than directed changes to alleles that favor plant vigor. Directional selection for increased vigor or forage yield of spaced plants is not routinely effective at increasing forage yield of sward plots. Selection for increased spaced-plant forage yield resulted in no change or a decrease in sward-plot forage yield for tall fescue, smooth brome-grass, reed canarygrass, and perennial ryegrass, *Lolium perenne* L. (Casler et al., 1996), and an increase in sward-plot forage yield for Italian ryegrass, *L. multiflorum* (Fujimoto and Suzuki, 1975), and Pensacola bahiagrass, *Paspalum notatum* Flüggé var. *saurae* Parodi (Gates et al., 1999). A number of studies have demonstrated heritability of spaced-plant forage yield, but the gains made under wide plant spacings in perennial ryegrass gradually diminished to zero as the plant spacing declined to sward-plot conditions (Hayward and Vivero, 1984). Taken as a whole, these results suggest that the correlation of spaced-plant vs. sward-plot forage yield is rarely of sufficient magnitude to make selection for increased forage yield of spaced plants a useful activity.

Selection responses in reed canarygrass indicated that P concentration has a moderate realized heritability of 0.26; that genetic gains are highly repeatable across harvests, locations, and years; and that there is a negative genetic

correlation between P concentration and sward-plot forage yield. The negative correlation was unexpected, based on phenotypic correlations observed in the original selection

Table 5. Means and *P* values for phosphorus concentration, forage yield, and phosphorus uptake of reed canarygrass populations created by divergent selection for P concentration of whole-plant herbage.

Variable, population	Harvest 1	Harvest 2	Harvest 3	Mean, total
P concentration [†]	g kg ⁻¹			
Low P	3.50	2.65	3.08	2.99
Original	3.55	2.77	3.26	3.09
High P	3.77	2.86	3.43	3.25
Linear <i>P</i> value	<0.0001	<0.0001	<0.0001	<0.0001
Forage yield [‡]	Mg ha ⁻¹			
Low P	4.00	4.24	1.75	9.71
Original	3.99	4.17	1.68	9.57
High P	3.43	3.90	1.58	8.65
Linear <i>P</i> value	<0.0001	0.0033	0.0031	<0.0001
P uptake [‡]	kg ha ⁻¹			
Low P	13.48	10.52	5.37	28.47
Original	13.77	10.62	5.56	29.03
High P	12.51	10.49	5.41	27.51
Linear <i>P</i> value	0.0051	0.9471	0.8269	0.0515

[†]Means over 2 yr, three locations, and eight replicates (means over three harvests in the last column).

[‡]Means over 2 yr, three locations, and eight replicates (totals over three harvests in the last column).

Table 6. Means and *P* values for N, K, Ca, and Mg concentration of reed canarygrass populations created by divergent selection for P concentration of whole-plant herbage.[†]

Variable, population	Harvest 1	Harvest 2	Harvest 3	Mean
N concentration	g kg ⁻¹			
Low P	26.9	21.1	26.1	23.3
Original	27.1	20.9	27.1	23.4
High P	27.9	21.6	27.3	24.0
Linear <i>P</i> value	0.0036	0.1403	<0.0001	0.0036
K concentration	g kg ⁻¹			
Low P	24.3	15.3	11.5	18.0
Original	25.2	15.8	12.8	18.8
High P	27.0	16.8	14.3	19.9
Linear <i>P</i> value	<0.0001	<0.0001	<0.0001	<0.0001
Ca concentration	g kg ⁻¹			
Low P	3.10	2.42	3.01	2.68
Original	3.18	2.40	3.13	2.70
High P	3.32	2.46	3.08	2.75
Linear <i>P</i> value	<0.0001	0.2505	0.0002	0.0017
Mg concentration	g kg ⁻¹			
Low P	2.58	1.98	2.46	2.23
Original	2.55	1.89	2.51	2.18
High P	2.72	1.94	2.45	2.23
Linear <i>P</i> value	0.0001	0.1724	0.6463	0.9873

[†]Means over 2 yr, three locations, and eight replicates.

Table 7. Means and *P* values for N, K, Ca, and Mg uptake of reed canarygrass populations created by divergent selection for P concentration of whole-plant herbage.[†]

Variable, population	Harvest 1	Harvest 2	Harvest 3	Total
N uptake				
	Mg ha ⁻¹			
Low P	102.0	78.4	41.5	215.0
Original	102.2	75.1	42.1	212.4
High P	89.3	74.8	39.0	196.7
Linear <i>P</i> value	0.0003	0.2042	0.1374	0.0003
K uptake				
	Mg ha ⁻¹			
Low P	97.9	68.2	21.1	183.7
Original	101.3	68.9	22.1	188.6
High P	91.9	68.5	22.5	179.1
Linear <i>P</i> value	0.0217	0.8866	0.1028	0.2127
Ca uptake				
	Mg ha ⁻¹			
Low P	11.45	9.67	5.06	25.34
Original	11.56	9.20	4.96	24.89
High P	10.28	8.99	4.59	23.10
Linear <i>P</i> value	0.0002	0.0062	0.0151	<0.0001
Mg uptake				
	Mg ha ⁻¹			
Low P	9.63	8.32	4.17	21.42
Original	9.34	7.81	3.99	20.48
High P	8.62	7.56	3.62	19.19
Linear <i>P</i> value	0.0001	0.0109	0.0003	<0.0001

[†]Means over 2 yr, three locations, and eight replicates.

nursery ($r = -0.06$ to 0.14). There appears, however, to be a fairly strong negative correlation between P concentration and forage yield in timothy (Bélanger and Richards, 1998; Bélanger et al., 2002). Similarly, differences among Italian ryegrass cultivars in total P extraction from the soil were unrelated to differences in herbage P concentration (Sistani et al., 2003), suggesting that forage yield was more important in regulating the total amount of P extracted during the season. The strong influence of forage yield on P uptake in reed canarygrass resulted in reduced P uptake in the high-P reed canarygrass population. The high-P population meets our goal of developing P-enriched forages for recycling P resources on farm but fails to meet the goal of “mining” more P from the soil.

The coincidence of positive correlated selection responses for N, K, Ca, and Mg concentration suggests that a common mechanism is likely responsible for the increase of all mineral elements in the high-P population relative to the low-P population. Possible mechanisms might be pleiotropy, in which the uptake of each element is regulated by common genes; linkage, in which genes regulating uptake of each element are coincidentally linked together; or concentration of mineral elements in a reduced herbage pool due to the reduction in dry-matter yield of the high-P population.

Physiological mechanisms for increasing P uptake include increasing frequency and/or surface area of root

hairs (Jungk, 2001; Wissuwa, 2003), increasing or modifying organic acid exudates from roots (Ming et al., 2002; Ström et al., 2002), or creating more efficient biological interdependences between host plants and arbuscular mycorrhizal fungi (Yao et al., 2001). Each of these mechanisms is under genetic control and could also function to increase uptake of additional mineral elements, suggesting that uptake of some mineral elements may share common inheritance mechanisms. Linkage is also likely, as there appear to be many genes involved in uptake of the major mineral elements (Beebe et al., 2006; Lin et al., 2004), and it is likely that both natural selection and plant breeding have concentrated favorable alleles for some of these genes in improved cultivars and populations. On the other hand, genes regulating uptake of P, Ca, and S were all mapped to different linkage groups in *Miscanthus sinensis* Anderss. (Atienza et al., 2003).

Finally, dilution may be a factor, but it cannot explain the coincidence of changes in concentration of these mineral elements alone. There was evidence for a reduction in uptake of all elements in the high-P population, suggesting that one or more active mechanisms of mineral uptake was impaired by the reduction in forage yield. It seems more likely that the high-P population has experienced a reduction in root mass, root exudate activity, root branching, root hair frequency, or some other uptake mechanism(s). Impairment of the P uptake system has likely resulted in reduced vigor and forage yield, reducing overall uptake of all elements measured in this study and concentrating smaller total amounts of nutrients into a smaller forage plant.

These results are in direct contrast to a wide array of traits that measure other aspects of forage nutritional value. Most plant traits that relate to digestibility, intake, and palatability, such as alkaloids, lignin, phenolic acids, fiber components, protein, and physical measures of cell-wall breakdown or leaf shear strength are less susceptible to the problems we encountered in this study (Casler, 2001). Most of these traits have a long history of genetic improvement using spaced plantings, single-harvest evaluations, and visual or cursory evaluations of plant vigor in many species of perennial forage crops (Casler, 2001). In general, selection for increased levels of these traits results in heritable gains that are repeatable across a wide range of environmental conditions (locations, years, harvests, managements) and are typically accomplished without significant reductions in forage yield (neutral detergent fiber concentration being the only prominent exception to the last point; Casler, 2001, 2005). That P concentration does not behave in a manner similar to these other nutritional-value traits is a strong warning that breeding procedures for increasing P concentration and uptake must be modified to improve the probability of success.

In conclusion, we demonstrated that P concentration is heritable in reed canarygrass and that NIRS technology can be used to predict P concentration in large breeding nurseries, with realized gains observed in sward-plot trials. We also showed that simple selection for increased P concentration was insufficient to increase P uptake, because P uptake was more a function of forage yield potential than P concentration per se. We demonstrated that estimates of genetic variability within the original selection nursery may be effective in predicting relative selection responses. We also conclude that future selection efforts must be based on genotypic selection methods, in which half-sib families are evaluated for forage yield, P concentration, and P uptake in sward plots. While these breeding methods are more expensive, time-consuming, and labor intensive, our data suggest that they will provide the only reliable method of breeding for increased P concentration and P uptake until effective DNA markers are developed.

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